

**Determination of the anti-SARS-CoV-2 efficacy of Anti-Microbe, Bio-Odeur and
PER OXY Activated solutions**

Experiments conducted under the supervision of

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INTRODUCTION.

SARS-CoV-2 is a Coronavirus responsible for the 2020 pandemic. As of October 2020, the virus has infected more than 40 million people and killed more than 1.1 million individuals. Stringent measures are needed to minimize the virus' propagations. Bio-Expert mandated Dr Flamand to test whether the Bio-Odeur, Anti-Microbe and PER OXY Activated solutions were effective in destroying SARS-CoV-2 infectivity.

MATERIALS AND METHODS

Experiments were conducted in the BSL-3 facility at the Centre de Recherche du CHU de Quebec. Isabelle Dubuc performed the experiments under the supervision of Dr Louis Flamand.

SARS-CoV-2 was obtained from the Laboratoire Santé Public du Quebec. The virus was propagated on Vero cells. The viral stock had an infectious titer of $1,2 \times 10^6$ TCID₅₀/mL.

Bio-Odeur, Anti-Microbe and PER OXY Activated solutions were provided by Bio-Expert.

Vero cells were plated in the wells of flat bottom 96-well plates (2×10^4 cells/well) in 200 μ l of culture medium the prior of the test.

To assess the cellular toxicity of the solutions, 100 μ l of medium was added to 900 μ l of undiluted solutions followed by serial dilutions in medium. The culture medium of the wells of the 96 well plates was removed and replaced with 200 μ l of serially diluted (in quadruplicates) solutions.

To assess the antiviral activity, 100 μ l of virus stock were added to 900 μ l of undiluted solutions for three minutes followed by serial dilutions in medium. The culture medium of the wells of the 96 well plates was removed and replaced with 200 μ l (in quadruplicates) of serially diluted virus preparations.

The plates were incubated for three days at 37°C, 5% CO₂. Each well was observed under the microscope to assess toxicity and infectivity.

Results

Assessment of compounds cellular toxicity. All three solutions were serially diluted in culture medium and added to Vero cells to assess toxicity. Concentrations of PER OXY

Activated solution above or equal to 0.3% (of the stock solution) were toxic to cells (Table 1).

Table 1: Toxicity assay of PER OXY Activated solution.

| Toxicity of PER OXY Activated solution (%) | | | | | | | | |
|--|--------|--------|--------|--------|--------|--------|--------|--------|
| 3,0000 | 0,9000 | 0,3000 | 0,0900 | 0,0300 | 0,0090 | 0,0030 | 0,0009 | medium |
| T | T | - | - | - | - | - | - | - |
| T | T | T | - | - | - | - | - | - |
| T | T | T | - | - | - | - | - | - |
| T | T | T | - | - | - | - | - | - |

Toxic (T) Non-toxic (-)

Concentrations of Anti-Microbe (Table 2) and Bio-Odeur (Table 3) solutions above or equal to 0.09% (of the stock solution) were toxic to cells.

Table 2: Toxicity assay of Anti-Microbe solution.

| Toxicity of Anti-Microbe solution (%) | | | | | | | | |
|---------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| 3,0000 | 0,9000 | 0,3000 | 0,0900 | 0,0300 | 0,0090 | 0,0030 | 0,0009 | medium |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |

Toxic (T) Non-toxic (-)

Table 3: Toxicity assay of Bio-Odeur solution.

| Toxicity of Bio-Odeur solution (%) | | | | | | | | |
|------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| 3,0000 | 0,9000 | 0,3000 | 0,0900 | 0,0300 | 0,0090 | 0,0030 | 0,0009 | medium |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |

Toxic (T) Non-toxic (-)

Assessment of compounds anti-SARS-CoV-2 activity

Concentration of PER OXY Activated solution >0.3% were toxic to the cells. As a result, it was not possible to assess infectivity in solutions carrying $\geq 0.3\%$ of the solution. Below this concentration no toxicity was observed. Results indicate that at the first testable dilution (0.09%), no residual signs of SARS-CoV-2 infectivity were recorded.

Table 4. Infectivity of SARS-CoV-2 after a three-minute incubation with PER OXY Activated solution.

| SARS-CoV-2 + PER OXY Activated solution | | | | | | | | medium |
|--|------|------|-------|-------|-------|-------|-------|--------|
| Virus dilution equivalent (relative to original viral stock) | | | | | | | | |
| 300 | 1000 | 3000 | 10000 | 30000 | 1E+05 | 3E+05 | 1E+06 | |
| T | T | - | - | - | - | - | - | - |
| T | T | - | - | - | - | - | - | - |
| T | T | - | - | - | - | - | - | - |
| T | T | - | - | - | - | - | - | - |

Toxic (T) No infection (-)

Concentration of Anti-Microbe and Bio-Odeur solution >0.09% were toxic to the cells. As a result, it was not possible to assess infectivity in solutions carrying $\geq 0.09\%$ of the solution. Below this concentration no toxicity was observed. Results indicate that at the first testable dilution (0.03%) of Anti-Microbe (Table 5) and Bio-Odeur (Table 6) no residual signs of SARS-CoV-2 infectivity were recorded.

Table 5. Infectivity of SARS-CoV-2 after a three-minute incubation with Anti-Microbe solution.

| SARS-CoV-2 + Anti-Microbe solution | | | | | | | | medium |
|--|------|------|-------|-------|-------|-------|-------|--------|
| Virus dilution equivalent (relative to original viral stock) | | | | | | | | |
| 300 | 1000 | 3000 | 10000 | 30000 | 1E+05 | 3E+05 | 1E+06 | |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |

Toxic (T) No Infection (-)

Table 6. Infectivity of SARS-CoV-2 after a three-minute incubation with Bio-Odeur solution.

| SARS-CoV-2 + Bio-Odeur solution | | | | | | | | |
|--|------|------|-------|-------|-------|-------|-------|--------|
| Virus dilution equivalent (relative to original viral stock) | | | | | | | | |
| 300 | 1000 | 3000 | 10000 | 30000 | 1E+05 | 3E+05 | 1E+06 | medium |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |
| T | T | T | T | - | - | - | - | - |

Toxic (T) No Infection (-)

Table 7. Titration of SARS CoV-2 viral stock.

| SARS-CoV-2 viral stock dilutions | | | | | | | | | |
|----------------------------------|------|------|-------|-------|-------|-------|-------|-------|--------|
| 300 | 1000 | 3000 | 10000 | 30000 | 1E+05 | 3E+05 | 1E+06 | 3E+06 | medium |
| + | + | + | + | + | + | + | + | - | - |
| + | + | + | + | + | - | + | - | - | - |
| + | + | + | + | + | + | - | - | - | - |
| + | + | + | + | + | + | - | - | - | - |
| + | + | + | + | + | + | - | + | - | - |
| + | + | + | + | + | + | - | - | - | - |
| + | + | + | + | + | + | - | - | - | - |
| + | + | + | + | + | + | - | - | - | - |

Infection (+) No infection (-)

Conclusion.

A three-minute incubation of SARS-CoV-2 with PER OXY Activated, Anti-Microbe or Bio-Odeur solutions were sufficient to destroy SARS-CoV-2 infectivity. However, the toxicity of the solutions prevented us from measuring with precision the quantity of virus killed by the solutions. While dilutions of 1:30 000 of the SARS-CoV-2 viral stock caused infection in 100 % of the wells tested (Table 7), at an identical virus dilution, no signs of infection were recorded for SARS-CoV-2 treated with PER OXY Activated, Anti-Microbe or Bio-Odeur solutions.